



TITLE:

Differences in hydrophyte life forms induce spatial heterogeneity of CH<sub>4</sub> production and its carbon isotopic signature in a temperate bog peatland

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## RESEARCH ARTICLE

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### Key Points:

- Hydrophyte life-forms affect much on CH<sub>4</sub> production on a temperate peat bog
- CH<sub>4</sub> concentration and  $\delta^{13}\text{C-CH}_4$  showed high heterogeneity among vegetation types
- CH<sub>4</sub> emissions differed even between the *Sphagnum* species

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# Differences in hydrophyte life forms induce spatial heterogeneity of CH<sub>4</sub> production and its carbon isotopic signature in a temperate bog peatland

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**Abstract** To clarify the effect of differences in hydrophyte life forms on methane (CH<sub>4</sub>) production and its carbon stable isotopic signature ( $\delta^{13}\text{C-CH}_4$ ), we analyzed CH<sub>4</sub> and carbon dioxide (CO<sub>2</sub>) concentrations, their stable carbon isotope values, and chemical constituents dissolved in pore water in a small floating peat bog in Japan. Because eutrophication has modified the surrounding water quality, the bog vegetation on the mat has been, in part, replaced by fen-type vegetation. We hypothesized that differences in hydrophyte habitats affect redox conditions, including dissolved oxygen (DO) in water and therefore the amounts and carbon isotopic values of CH<sub>4</sub> and CO<sub>2</sub> dissolved in pore water. Between the habitats of two *Sphagnum* species, DO was considerably higher, and CH<sub>4</sub> concentrations were significantly lower in *Sphagnum cuspidatum* Ehrh. habitats in hollow (DO:  $0.62 \pm 0.20$  mg/L (standard error (SE)) and CH<sub>4</sub>:  $0.18 \pm 0.02$  mmol/L) than in *Sphagnum palustre* L. habitats in hummock (DO:  $0.29 \pm 0.08$  and CH<sub>4</sub>:  $0.82 \pm 0.06$ ) in pore water (10 cm depth). Both DO and CH<sub>4</sub> concentrations in three vascular plant habitats (*Rhynchospora fauriei* Franch., *Phragmites australis* [reed], and *Menyanthes trifoliata* L.) in pore water (10 cm depth) were intermediate relative to the two *Sphagnum* species. However, CH<sub>4</sub> flux in *M. trifoliata* site was significantly higher than that at both *Sphagnum* sites, suggesting that the type of gas transport (diffusive or convective via root and stem) affected the depth profile of CH<sub>4</sub> concentrations and its flux.  $\delta^{13}\text{C-CH}_4$  values in pore water also varied among the vegetation types, even within *Sphagnum* species (e.g., at 10 cm depth,  $\delta^{13}\text{C-CH}_4$ : *R. fauriei*,  $-55.3 \pm 1.8\text{‰}$  (SE); *P. australis*,  $-57.5 \pm 1.6\text{‰}$ ; *M. trifoliata*,  $-56.7 \pm 1.5\text{‰}$ ; *S. cuspidatum*,  $-71.2 \pm 1.4\text{‰}$ ; and *S. palustre*,  $-60.4 \pm 0.6\text{‰}$ ). Our results suggest that significant differences arise in CH<sub>4</sub> concentration and  $\delta^{13}\text{C-CH}_4$  values among the hydrophyte habitats even within a small peat bog and that change in vegetation relative to trophic conditions can affect CH<sub>4</sub> emissions and associated  $\delta^{13}\text{C-CH}_4$  values.

## 1. Introduction

Methane (CH<sub>4</sub>) is a key greenhouse gas (GHG) that has an infrared radiative heating effect 28 to 34 times greater than that of carbon dioxide (CO<sub>2</sub>) on a mass basis over a 100 year time horizon [Intergovernmental Panel on Climate Change (IPCC), 2013]. Based on the inversion of atmospheric measurements of CH<sub>4</sub> from surface stations, global CH<sub>4</sub> emissions for the 2000s are 553 Tg CH<sub>4</sub> yr<sup>-1</sup>, with a range of 526–569 Tg CH<sub>4</sub> yr<sup>-1</sup> [IPCC 2013]. The contribution from natural CH<sub>4</sub> sources (i.e., wetlands, oceans, geological seepage, termites, and vegetation) is estimated at 215 Tg CH<sub>4</sub> [Schlesinger and Bernhardt, 2013]. In anoxic environments such as wetland soils, CH<sub>4</sub> is produced by methanogenic archaea that are active only under anoxic and strongly reducing conditions [Takai, 1970; Schütz et al., 1989]. In contrast, CH<sub>4</sub> in oxic soils is usually oxidized by methanotrophic bacteria. Improved estimates of the strengths of the various CH<sub>4</sub> sources and sinks are of high importance, as although most sources and sinks of CH<sub>4</sub> have been identified, their relative contributions to atmospheric CH<sub>4</sub> levels are still uncertain [Kirschke et al., 2013].

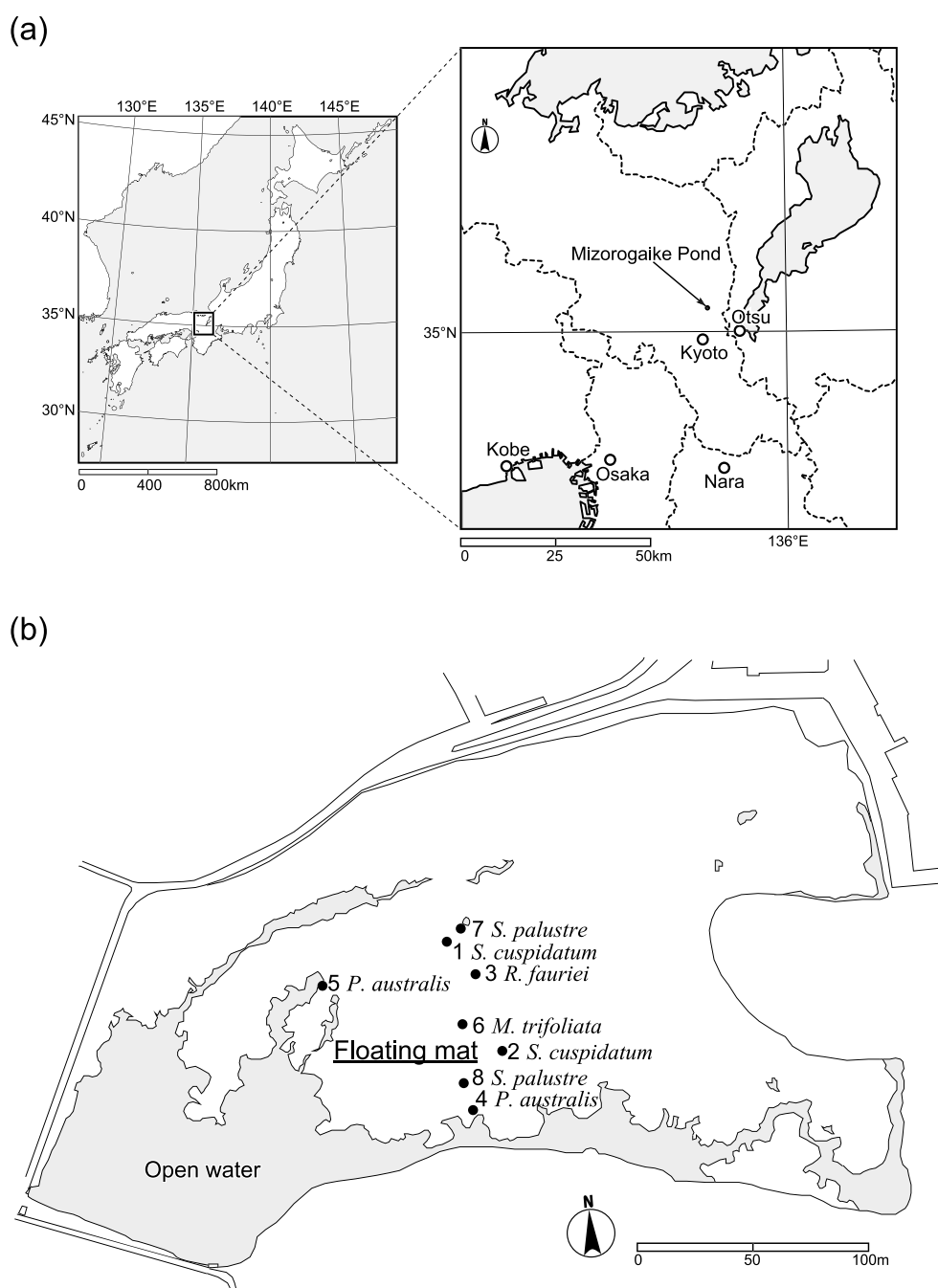
Among the major natural sources, the single most dominant CH<sub>4</sub> source of the global flux and interannual variability is CH<sub>4</sub> emissions from wetlands (177 to 284 Tg CH<sub>4</sub> yr<sup>-1</sup>) [IPCC, 2013], and many studies have revealed that various environmental parameters affect CH<sub>4</sub> emissions from natural wetlands, including peatlands, such as soil characteristics, water table depth, and soil temperature (reviewed in Topp and Pattey [1997]). However, estimates of global CH<sub>4</sub> emissions from wetlands are not well constrained. The lack of information on the effects of vegetation types on wetlands is likely

one of the reasons for the uncertainty [Carmichael *et al.*, 2014]. Because wetland trophic status affects CH<sub>4</sub> production and emission rates by controlling vegetation assemblages [Whiting and Chanton, 1993], several studies were conducted to understand the effect of trophic status on CH<sub>4</sub> dynamics. Many of them revealed that the CH<sub>4</sub> flux was higher in minerotrophic peatlands than in ombrogenous bogs [Kelley *et al.*, 1992; Chanton *et al.*, 1995; and Bellisario *et al.*, 1999; Hornibrook and Bowes, 2007]. Some studies have focused on vegetation types and cover [King *et al.*, 1998; Van der Nat and Middelburg, 2000; Joabsson and Christensen, 2001; Ström *et al.*, 2005], addressing the effects of vascular plants on CH<sub>4</sub> emission. However, studies examining various types of hydrophyte cover in wetlands including *Sphagnum* species are not enough [e.g., Sugimoto and Fujita, 1997, 2006]. Considering that nutrient condition affects the vegetation cover (e.g., a greater abundance of *Sphagnum* spp. in nutrient-poor acidic ombrogenous bogs) and eutrophication can cause changes in vegetation type [Haraguchi and Matsui, 1990; Shimamura *et al.*, 2006], more detailed information is required regarding CH<sub>4</sub> dynamics in typical vegetation covers.

Because biospheric sources of CH<sub>4</sub> are highly variable, stable isotope ratios of CH<sub>4</sub> have been used to constrain the global CH<sub>4</sub> budget, as microbe-produced CH<sub>4</sub> has a significantly different isotopic signal than CH<sub>4</sub> from other sources [Whiticar, 1999]. In particular, the stable carbon isotopic compositions of CH<sub>4</sub> ( $\delta^{13}\text{C-CH}_4$ ) in background tropospheric air and the major sources of CH<sub>4</sub> have further constrained the individual CH<sub>4</sub> source strengths through the isotope mass balance method, using the  $\delta^{13}\text{C}$  value of each source [Bräunlich *et al.*, 2001; Fletcher *et al.*, 2004; reviewed in Dlugokencky *et al.*, 2011]. However, estimating the representative  $\delta^{13}\text{C-CH}_4$  of each source remains challenging because  $\delta^{13}\text{C-CH}_4$  values are highly variable, especially in rice paddies and wetlands [Quay *et al.*, 1991], reflecting the multiple processes involved in CH<sub>4</sub> production, consumption, and transport via plants in these ecosystems. For example, methanogenesis from carbonate results in a larger fractionation against  $^{13}\text{C}$  and, thus, more negative  $\delta^{13}\text{C-CH}_4$  values than methanogenesis from acetate [Games *et al.*, 1978; Krzycki *et al.*, 1987; Gelwicks *et al.*, 1994]. Previous studies have shown that the fractionation factors vary depending on location and conditions (e.g., reviewed by Conrad [2005]). Several studies have reported that acetoclastic methanogenesis is suppressed in some peat ecosystems and that CH<sub>4</sub> from carbonate reduction (H<sub>2</sub>/CO<sub>2</sub>) is the dominant pathway [Lansdown *et al.*, 1992; Horn *et al.*, 2003; Metje and Frenzel, 2005; Prater *et al.*, 2007]. Galand *et al.* [2005] reported that the highest portion of CH<sub>4</sub> from H<sub>2</sub>/CO<sub>2</sub> was observed from oligotrophic fen followed by the ombrotrophic bog, and the lowest was observed from the mesotrophic fen based on community studies of methanogen. The same pattern was supported by pore water  $\delta^{13}\text{C-CH}_4$  values, which were higher in minerotrophic peatland than in ombrogenous peatland. [Hornibrook and Bowes, 2007]. However, to study the shift in  $\delta^{13}\text{C-CH}_4$  values with change in trophic status, data from various vegetation types on a single peatland can add useful information.

Especially, information on the effects of different *Sphagnum* habitats is totally lacking, although their life forms and decomposition patterns are species specific. For example, the rate of decomposition of *Sphagnum* in hollows (including *Sphagnum cuspidatum*) was higher than those of *Sphagnum* in hummocks [Hogg, 1993], and Johnson and Damman [1991] suggested that the decomposition rate was faster in *S. cuspidatum* in hollows than in *S. fuscum* in hummocks. In addition, *Sphagnum* mosses were shown to be able to oxidize CH<sub>4</sub> through symbioses with partially endophytic methanotrophic bacteria [Raghoebarsing *et al.*, 2005]. For this CH<sub>4</sub> oxidation, Kip *et al.* [2010] found that all mosses collected from pools, lawns, and hummocks were capable of oxidizing CH<sub>4</sub> and that the rate of CH<sub>4</sub> oxidation was most pronounced in submerged mosses (pools in hollows). These results suggest that differences in the dominant *Sphagnum* species are indicative of the various CH<sub>4</sub> dynamics in peat mats.

Therefore, we postulate that vegetative heterogeneity in peatlands assures heterogeneity in CH<sub>4</sub> dynamics. If differences occur in CH<sub>4</sub> production pathways, oxidation, and transport processes among the habitats, the carbon isotope signatures of the CH<sub>4</sub> produced should differ. This must be considered to obtain a better understanding of CH<sub>4</sub> dynamics. In this study, we used the  $\delta^{13}\text{C}$  signatures of pore water CH<sub>4</sub> and CO<sub>2</sub> in a temperate peatland to determine differences in CH<sub>4</sub> dynamics at the sample sites to understand the processes that control both CH<sub>4</sub> concentrations and carbon isotopic values.

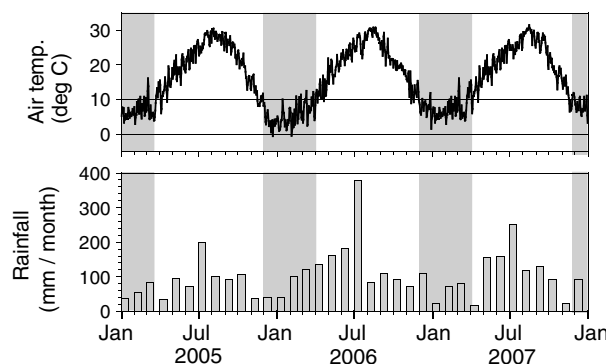


**Figure 1.** (a) Locations of Kyoto City and Mizorogaike Pond. (b) Study sites on the floating mat in Mizorogaike Pond. Circles show the sampling points and habitat types.

## 2. Materials and Methods

### 2.1. Site Description and Overview of the Preceding Biogeochemical Observations at the Site

Mizorogaike Pond (35°03'N, 135°50'E; 75 m asl) is a natural pond (0.08 km<sup>2</sup> in area and 1 km in circumference) located in the northern area of Kyoto City, Japan (Figures 1a and 2). This system contains a floating mat, or bog, in the center of the pond consisting of about 0.05 km<sup>2</sup> of peat (floating area, 0.03 km<sup>2</sup>) on which mire vegetation occurs, including some relic species from the last ice age [Haraguchi and Matsui, 1990; Haraguchi, 1991]. The mat edges are connected with the open water. Miki [1929] noted that this floating



**Figure 2.** Seasonal variations in air temperature and precipitation in Kyoto City.

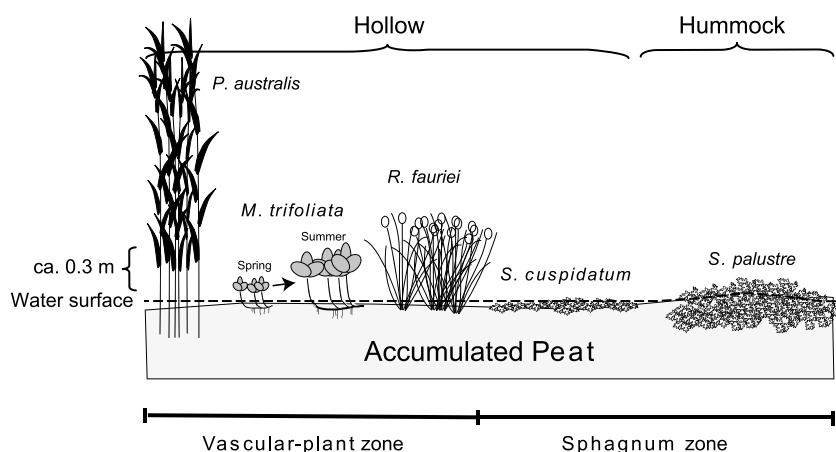
mat was oligotrophic and entirely covered with *Sphagnum* bog. During the past few decades, however, the *Sphagnum* cover has largely been degraded, and *Phragmites australis* has been invading this floating bog from the periphery of the pond. This means that the bog vegetation has been replaced, in part, by fen-type vegetation, possibly because of eutrophication of the surrounding open water [Haraguchi and Matsui, 1990; Shimamura et al., 2006]. The bog on the floating mat is composed of several microtopographies, such as hummocks, hollows, and pools. About 130 hummocks are included in the area of 0.2–200 m<sup>2</sup> [Shimizu, 1986].

The floating mat was about 1.2–1.8 m thick and contained two species of *Sphagnum*: *S. cuspidatum* Ehrh., which inhabited hollows, and *Sphagnum palustre* L., which occurred on hummocks. *S. cuspidatum* and other emergent plants were patchily distributed throughout the hollow sections of the mat [Investigation Group for Mizorogaike Pond in the Research Institute of Plant Biology in Kyoto University, 1981].

Although the bog is floating by gases such as CO<sub>2</sub> and CH<sub>4</sub> derived from the decomposition of organic matter and it shows floating (summer)-sinking (winter) movement compared with the open-water surface level, the amplitude of water-level fluctuation in the location of each hydrophyte community is within 0.1 m throughout the year in most parts of the hollow [Haraguchi, 1991].

Bare hollows did not contain *S. cuspidatum* and were dominated by *Menyanthes trifoliata* L., which was the most dominant species in the mat, except in the hummocks. The *Sphagnum*-rich hollows, which contained *S. cuspidatum*, were distributed along the north side of the floating mat. Some communities of *Rhynchospora fauriei* Franch. also occur in hollows. Habitats of *P. australis* were seen at mat edges that bordered the open water and were slightly flooded. We categorized the typical habitats of the floating mat into five types, described in the next section (Figures 1b and 3).

Haraguchi [1991] investigated the relationship between vegetation and seasonal water-level changes in Mizorogaike Pond, except on the hummocks and suggested that *S. cuspidatum* favors stable water-level conditions that fluctuate near the level at which the community can be submerged. In contrast, the hummocks consist of *S. palustre* and some shrub species that were scattered throughout the mat [Shimizu, 1986]. Haraguchi and Matsui [1990] investigated the chemical properties of water in the hollows and reported that water around *S. cuspidatum* communities had lower pH and electrical conductivity (EC) values than that around *M. trifoliata* communities.



**Figure 3.** Schematics of cross-sectional diagram of habitat types on the floating mat in Mizorogaike Pond (summer period).

*Sugimoto and Fujita* [1997] observed  $\text{CH}_4$  flux and  $\delta^{13}\text{C}$  of dissolved and bubble  $\text{CH}_4$  below the ground surface in Mizorogaike Pond. Their observation were made at three sites with the typical vegetation and characteristic seasonal water-level cycles of the floating mat: (1) a reed site that consisted of *P. australis*; (2) a marsh trefoil site that consisted of *M. trifoliata*; and (3) a *Sphagnum* site that consisted of both *S. cuspidatum* and *S. palustre*. They reported that the averaged  $\text{CH}_4$  fluxes from April to October (spring to fall) were highest at the marsh trefoil site ( $600 \text{ mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$ ) followed by the reed site ( $387 \text{ mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$ ), and the smallest flux was at the *Sphagnum* site ( $93 \text{ mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$ ), reflecting the differences in the decomposability of organic matter.  $\delta^{13}\text{C}\text{-CH}_4$  values obtained from pore water at 10 cm depth in their study were  $-64.9$  to  $-52.8\text{‰}$  at the reed site,  $-65.0$  to  $-52.5\text{‰}$  at the marsh trefoil site, and  $-61.5$  to  $-54.0\text{‰}$  at the *Sphagnum* site. *Sugimoto and Fujita* [2006] also reported that the concentration of hydrogen ( $\text{H}_2$ ) in bubble gas in the floating mat that was available for  $\text{CH}_4$  production through carbonate reduction increased in summer. These reports provide valuable information on  $\text{CH}_4$  dynamics in this peatland; however, the two *Sphagnum* species (*S. cuspidatum* and *S. palustre*) were treated as one community with similar characteristics. As we mentioned previously, a more precise understanding of  $\text{CH}_4$  dynamics can be obtained by treating the two *Sphagnum* species separately. Considering their life forms and the rate of decomposition of *Sphagnum*, because it was expected to be higher in hollows (including *S. cuspidatum*) than in hummocks [Hogg, 1993], Johnson and Damman [1991] suggested that decomposition rate was faster in *S. cuspidatum* in hollows than in *S. fuscum* on hummocks.

## 2.2. Distribution of Plant Species and Sampling Points

Figure 3 shows the schematics of the cross-sectional diagram of habitat types on the floating mat in Mizorogaike Pond in summer. We collected pore water samples from five major hydrophyte habitats on the floating mat.

1. *S. cuspidatum* *S. cuspidatum* is able to grow at the transition between lawn and hummock and can be found growing in the open water, completely submerged. Haraguchi [1991] reported that the greatest number of capitula of *S. cuspidatum* was observed in areas where the water level fluctuations were small and flooding occurred for only a short period. We designated two sampling points as *S. cuspidatum*-dominated sites. One site was located at Point 1 in the northern part of the floating mat and the second was located at Point 2 in the southern part (Figure 1b).
2. *R. fauriei* (vascular) *R. fauriei* grows in bare hollows at the center of the floating mat in Mizorogaike Pond. The distribution of this species is limited to habitats that experience flooding in winter [Haraguchi 1991]. We observed this species at one sampling point (Point 3) in the northern part of the floating mat (Figure 1b).
3. *P. australis* (reed and vascular) *P. australis* is dominant at the margins of the mat. Shoots of *P. australis* open twice per year (June and September). These reed sites are submerged throughout the year, because the roots of the reeds reach the bottom of the pond and anchor the floating mat. We selected two sampling points at the margins of the mat. One was located at Point 4 on the southern edge of the mat and the other was at Point 5 on western edge (Figure 1b).
4. *M. trifoliata* (vascular) *M. trifoliata* is a clonal plant with a horizontal creeping sympodial rhizome with adventitious roots and leaves at the apex. Live rhizomes of *M. trifoliata* cover almost the entire mat at a depth of approximately 5 cm [Haraguchi 1996]. It shoots twice per year in Mizorogaike Pond, the southern limit of its distribution. The first shoot emerges in mid-April and dies in July, and the second shoot grows from September to October. This species is tolerant of flooding [Coult and Vallance, 1958]. We chose one sampling point (Point 6) at the center of the mat (Figure 1b). Based on the results of Haraguchi [1991], on the Mizorogaike Pond floating mat, *M. trifoliata* is the main vegetation with larger water-level fluctuation (approximately 6 to 10 cm), and it prefers higher water-table conditions (0 to 12 cm) than does *S. cuspidatum*. Sugimoto and Fujita [1997] reported that fluctuation of water levels above the peat surface was less than 10 cm at their *M. trifoliata* site, which is also at the center of the mat and in a similar condition to our sampling point.
5. *S. palustre* *S. palustre* grows on small hummocks where the water table is below the surface of the mat throughout the year. We set the two sampling points on the hummocks where *S. palustre* plant tissue accumulated to more than 10 cm, and the water-table level was kept below the *Sphagnum* surface in these sites (Figure 3). The depth of the water level never dropped lower than 10 cm from the *Sphagnum* surface during our sampling period. This is probably because of capillary flow of water from the water table to the *Sphagnum* surface [Price, 1996] in addition to floating-sinking movement of the mat. One was located at Point 7 in northern part of the mat and the other was at Point 8 in the southern part (Figure 1b).



For the three species for which samples were collected at two sampling points, *S. cuspidatum*, *P. australis*, and *S. palustre*, we averaged the values of the two samples from each sampling day for analysis.

### 2.3. Water Sample Collection and Analyses

Pore water samples were collected vertically to measure dissolved gases and water chemistry. The pore water samplers were placed at depths of 10 and 40 cm from the soil or *Sphagnum* surface to compare to the results of the same depth sampling by Sugimoto and Fujita [1997]. We used a double-walled sampler screened with numerous vertically aligned 2 mm holes constructed by placing a 100 mL polypropylene bottle inside a wide-mouth, 200 mL polystyrene bottle [Itoh et al., 2007], which collected pore water without degassing or high decompression and could exclude soil and detritus. Pore water samples were injected into 20 or 30 mL preevacuated vials for the measurement of dissolved CH<sub>4</sub> and CO<sub>2</sub> concentrations and carbon isotope ratios without exposure to the atmosphere and into plastic bottles for other chemical analyses; the vials and plastic bottles were stored in a cooler (~4°C) in the field. In situ measurements, including pH and EC (from August 2005 to August 2006) and laboratory measurements of dissolved components including dissolved O<sub>2</sub> (DO), CH<sub>4</sub>, and CO<sub>2</sub> were conducted. For measurements of dissolved oxygen (DO), water samples were collected in 100 mL biological oxygen demand (BOD) bottles and then fixed immediately after sampling. DO was determined following the Winkler method. Measurements of DO were conducted from August 2005 to August 2006.

Samples for CH<sub>4</sub> and CO<sub>2</sub> concentrations and their carbon isotopic compositions ( $\delta^{13}\text{C-CH}_4$  and  $\delta^{13}\text{C-CO}_2$ ) were collected monthly at most sampling points and depths from August 2005 to August 2006. For Point 2, observations were performed from December 2005 to August 2006. Water samples for Points 1, 2, and 4 (for *S. cuspidatum*, *M. trifoliata*, and *S. palustre*, respectively, 10 cm depth) were also taken from October 2006 to December 2007 to compare the dissolved gas components with surface CH<sub>4</sub> fluxes. CH<sub>4</sub> concentrations were determined using a gas chromatograph (GC; GC-14BPF, Shimadzu, Japan) equipped with a flame ionization detector (FID) and a Porapack Q column (3 mm i.d. (inner diameter)  $\times$  2 m, Shinwa Chemical Industry, Japan) using N<sub>2</sub> (flow rate, 50 mL min<sup>-1</sup>) as the carrier gas. For dissolved CO<sub>2</sub> concentration measurements, samples of the same gas were collected from the headspace and injected into a GC (GC-8APT, Shimadzu, Japan) equipped with a thermal conductivity detector and a Shincarbon T column (2 mm i.d.  $\times$  6 m, Shinwa Chemical Industry, Japan) using He (flow rate, 50 mL min<sup>-1</sup>) as the carrier gas [Itoh et al., 2007]. Note that pore water samples were not acidified prior to analysis of dissolved CO<sub>2</sub> concentration and  $\delta^{13}\text{C-CO}_2$ .

Carbon isotopic compositions of dissolved CH<sub>4</sub> and CO<sub>2</sub> were analyzed using a gas chromatograph/combustion/isotope ratio mass spectrometer (Thermo Finnigan MAT252; Thermo Fisher Scientific, Waltham, MA, USA) equipped with an HP G1530A (Agilent, Santa Clara, CA, USA) GC system [Sugimoto, 1996] at the Center for Ecological Research at Kyoto University. CH<sub>4</sub> was separated on a PoraPLOT Q capillary column (0.32 mm i.d.  $\times$  25 m) and combusted to CO<sub>2</sub> at 940°C in a ceramic reactor containing CuO and Pt wires. The stable isotope ratios are expressed in the standard delta ( $\delta$ ) notation in units of per mil (‰) relative to Vienna Pee Dee Belemnite (VPDB). The analytical precision was better than  $\pm 0.2\text{‰}$  when 44 nmol of CO<sub>2</sub> or CH<sub>4</sub> was injected.

The water samples collected for dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) analysis were filtered through 0.45  $\mu\text{m}$  polytetrafluorethylene membrane filters and stored in glass vials at 4°C. DOC and TDN concentrations were measured using a total organic carbon analyzer with chemiluminescent detection of total nitrogen (TOC-V, TNM-1, Shimadzu, Japan). Samples for measurements of NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, and SO<sub>4</sub><sup>2-</sup> concentrations were filtered through 0.20  $\mu\text{m}$  cellulose acetate membrane filters and analyzed by ion chromatography (ICS-90, Dionex, Japan). Dissolved organic nitrogen (DON) was calculated as the difference between TDN and inorganic N (NH<sub>4</sub><sup>+</sup> + NO<sub>3</sub><sup>-</sup>).

We also collected rainwater from August 2005 to December 2006 using a rainfall collector installed in the center area of the floating mat that consisted of a 5.0 L polypropylene bottle, a polypropylene funnel (180 mm diameter), and a 1.0 mm mesh screen.

### 2.4. Methane Flux Measurements

We measured CH<sub>4</sub> flux at Points 1, 6, and 7 (*S. cuspidatum*, *M. trifoliata*, and *S. palustre*, respectively; Figure 1) from October 2006 to December 2007. Three polyvinyl chloride (PVC) static chambers (diameter: 0.26 m;

volume: 0.014 m<sup>3</sup>) were placed at each observation point. When measuring, the chamber top was placed gently on the chamber base and covered with aluminum foil to reduce heating during sampling. Samples of the chamber air were collected 4 times within 0.75 h from each chamber with a 50 mL syringe via the septum stopper on the chamber top. The samples were immediately injected into preevacuated 10 mL vials fitted with butyl rubber stoppers for laboratory analysis.

Analyses of CH<sub>4</sub> concentrations were conducted using GC-FID. The samples were always analyzed within 2 days of collection. The testing revealed that the vacuum inside the vials could be retained for a period of 1 week and that the sample integrity could also be maintained for the same duration. CH<sub>4</sub> flux was calculated based on the change in the headspace gas concentration over time, assuming first-order kinetics [Dobbie and Smith 1996]. In our observations, all analyses of individual time series revealed that slope values for CH<sub>4</sub> versus time were linear. At each sampling point, the flux was measured only once daily, and the measurements for each observation were performed at approximately the same time during the daytime.

### 2.5. Plant Tissue Sampling and Analysis

Plant samples were collected for *S. cuspidatum*, *M. trifoliata*, *S. palustre*, *R. fauriei*, and *P. australis* in December 2006. Plant samples of *S. cuspidatum*, *M. trifoliata*, and *S. palustre* were collected in quintuplicate.  $\delta^{13}\text{C}$  analyses were performed on aboveground sections of the plant tissues. The samples were dried in an oven at 40°C for 48 h prior to  $\delta^{13}\text{C}$  analysis.  $\delta^{13}\text{C}$  was determined using a mass spectrometer (Delta plus XP, Thermo Electron) coupled with an elemental analyzer (Flash EA, Thermo Electron). The standards were VPDB for  $\delta^{13}\text{C}$ . We used DL-alanine as a working standard. The analytical precision was better than  $\pm 0.2\text{‰}$  for  $\delta^{13}\text{C}$ . When differences in means were determined to be statistically significant, Tukey's honestly significant difference test was used.

## 3. Results

### 3.1. Environmental Conditions

Daily mean air temperatures and precipitation observed at Kyoto Weather Observatory (5 km southeast of Mizorogaike) are shown in Figure 2. Annual precipitation measurements for 2005, 2006, and 2007 were 954.5, 1582.5, and 1212.5 mm, respectively. Compared to the 10 year average (1373.1 mm for 2000 to 2009), 2006 was the rainiest year and 2005 was the driest during this 10 year period. It is noteworthy that the winters of 2005/2006 and 2006/2007 were the coldest (mean temperature from December to February: 4.5°C) and warmest (6.9°C), respectively, from 2001 to 2010 (10 year average mean temperature from December to February: 5.8°C).

Both pH and EC were lower at the *Sphagnum* sites (Table 1). EC was low in rainfall. The highest pH in pore water was 5.06 at the 10 cm depth at the edges of the mat (*P. australis* sites), suggesting that most dissolved carbonate species at all sampling sites existed as CO<sub>2</sub> under low pH conditions. NO<sub>3</sub><sup>−</sup> concentrations at all sampling sites were almost zero and no significant differences were identified in NO<sub>3</sub><sup>−</sup> (10 cm). Significantly higher NH<sub>4</sub><sup>+</sup> concentrations were observed at the *S. palustre* sites for both sampling depths compared to the other habitat types ( $t > 3.9$ ,  $p < 0.003$  for 10 cm depth;  $t > 5.7$ ,  $p < 0.0001$  for 40 cm depth) with the exception of the *M. trifoliata* site at the 10 cm depth ( $t = 1.8$ ,  $p = 0.40$ ). NH<sub>4</sub><sup>+</sup> concentrations at 10 cm at the *M. trifoliata* site were significantly higher than at the *S. cuspidatum* and *P. australis* sites ( $t > 2.9$ ,  $p < 0.047$ ). SO<sub>4</sub><sup>2−</sup> was significantly higher at the *S. cuspidatum* sites at 10 cm than at the *S. palustre* and *R. fauriei* sites ( $t > 3.2$ ,  $p < 0.022$ ). No significant differences were found in SO<sub>4</sub><sup>2−</sup> concentrations (40 cm) among all sampling sites. The DOC concentration was highest at the *M. trifoliata* site; however, no significant difference was found in DOC concentrations among the sites. The DON concentration was significantly lower at the *S. palustre* site at 10 cm depth than at the *M. trifoliata* and *P. australis* sites ( $t > 3.1$ ,  $p < 0.03$ ). At 40 cm depth, DON concentration was significantly higher at the *P. australis* site than at all the other sites ( $t > 3.4$ ,  $p < 0.01$ ).

In this study, we used the molar ratio of dissolved C to N (DOC/DON) in pore water to assess the degree of labile C because lower DOC/DON ratios are indicative of more labile organic matter [Melillo et al., 1982; Finzi et al., 1998; Corbett et al., 2013]. At the *S. palustre* site, the DOC/DON ratio was among the highest at both 10 cm depth ( $t > 3.1$ ,  $p < 0.022$ ) and 40 cm depth ( $t > 3.4$ ,  $p < 0.011$ ; Table 1). The  $\delta^{13}\text{C}$  values for plants were



**Table 1.** Mean ( $\pm$  SE) Values for pH, EC,  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ , DOC, DON Concentrations, and DOC/DON Ratio at Each Sampling Site and Depth

Plot	Depth (cm)	$\text{pH}$	$\text{EC}$ ( $\mu\text{S}/\text{cm}$ )	$\text{b}_{\text{NH}_4^+}$ (mg-N/L)	$\text{b}_{\text{NO}_3^-}$ (mg-N/L)	$\text{b}_{\text{SO}_4^{2-}}$ (mg-S/L)	$\text{a}_{\text{DOC}}$ (mmol/L)	$\text{b}_{\text{DON}}$ (mmol/L)	$\text{b}_{\text{DOC/DON}}$	N
Rainfall		$5.9 \pm 0.3$	$19.4 \pm 0.2$	NM	NM	NM	NM	NM	NM	25
<i>S. cuspidatum</i>	10	$4.5 \pm 0.0$	$35.9 \pm 2.7$	$0.24 \pm 0.12$	$0.00 \pm 0.00$	$0.25 \pm 0.10$	$2.05 \pm 0.31$	$0.05 \pm 0.00$	$37.3 \pm 4.1$	11
	40	$4.5 \pm 0.0$	$32.0 \pm 1.0$	$0.39 \pm 0.04$	$0.00 \pm 0.00$	$0.06 \pm 0.02$	$1.65 \pm 0.11$	$0.04 \pm 0.00$	$37.4 \pm 2.7$	11
<i>R. fauriei</i>	10	$4.8 \pm 0.1$	$43.2 \pm 5.6$	$0.43 \pm 0.21$	$0.00 \pm 0.00$	$0.03 \pm 0.01$	$2.18 \pm 0.19$	$0.06 \pm 0.01$	$38.0 \pm 5.7$	11
	40	$4.5 \pm 0.0$	$28.9 \pm 1.2$	$0.21 \pm 0.11$	$0.00 \pm 0.00$	$0.02 \pm 0.01$	$1.43 \pm 0.06$	$0.04 \pm 0.01$	$31.8 \pm 1.1$	11
<i>P. australis</i>	10	$5.1 \pm 0.1$	$56.3 \pm 7.1$	$0.20 \pm 0.13$	$0.00 \pm 0.00$	$0.12 \pm 0.02$	$2.36 \pm 0.32$	$0.07 \pm 0.01$	$31.4 \pm 3.9$	11
	40	$4.9 \pm 0.1$	$42.6 \pm 2.4$	$0.53 \pm 0.27$	$0.00 \pm 0.00$	$0.04 \pm 0.01$	$2.06 \pm 0.37$	$0.04 \pm 0.01$	$34.9 \pm 4.5$	11
<i>M. trifoliata</i>	10	$5.0 \pm 0.1$	$52.9 \pm 6.0$	$0.98 \pm 0.25$	$0.00 \pm 0.00$	$0.09 \pm 0.02$	$2.82 \pm 0.50$	$0.08 \pm 0.01$	$26.4 \pm 1.7$	11
	40	$4.6 \pm 0.1$	$48.7 \pm 7.6$	$0.49 \pm 0.07$	$0.00 \pm 0.00$	$0.06 \pm 0.01$	$2.93 \pm 1.08$	$0.08 \pm 0.01$	$27.8 \pm 4.4$	11
<i>S. palustre</i>	10	$4.7 \pm 0.1$	$34.0 \pm 1.8$	$1.43 \pm 0.17$	$0.01 \pm 0.00$	$0.04 \pm 0.01$	$2.17 \pm 0.17$	$0.03 \pm 0.01$	$75.4 \pm 17.6$	11
	40	$4.7 \pm 0.0$	$36.3 \pm 2.3$	$1.70 \pm 0.11$	$0.02 \pm 0.01$	$0.03 \pm 0.01$	$2.26 \pm 0.11$	$0.03 \pm 0.00$	$81.9 \pm 20.3$	11

<sup>a</sup>Samples for pH, EC, and DOC concentrations were obtained monthly from August 2005 to August 2006.

<sup>b</sup>Number of measurements was nine. "NM", not measured.

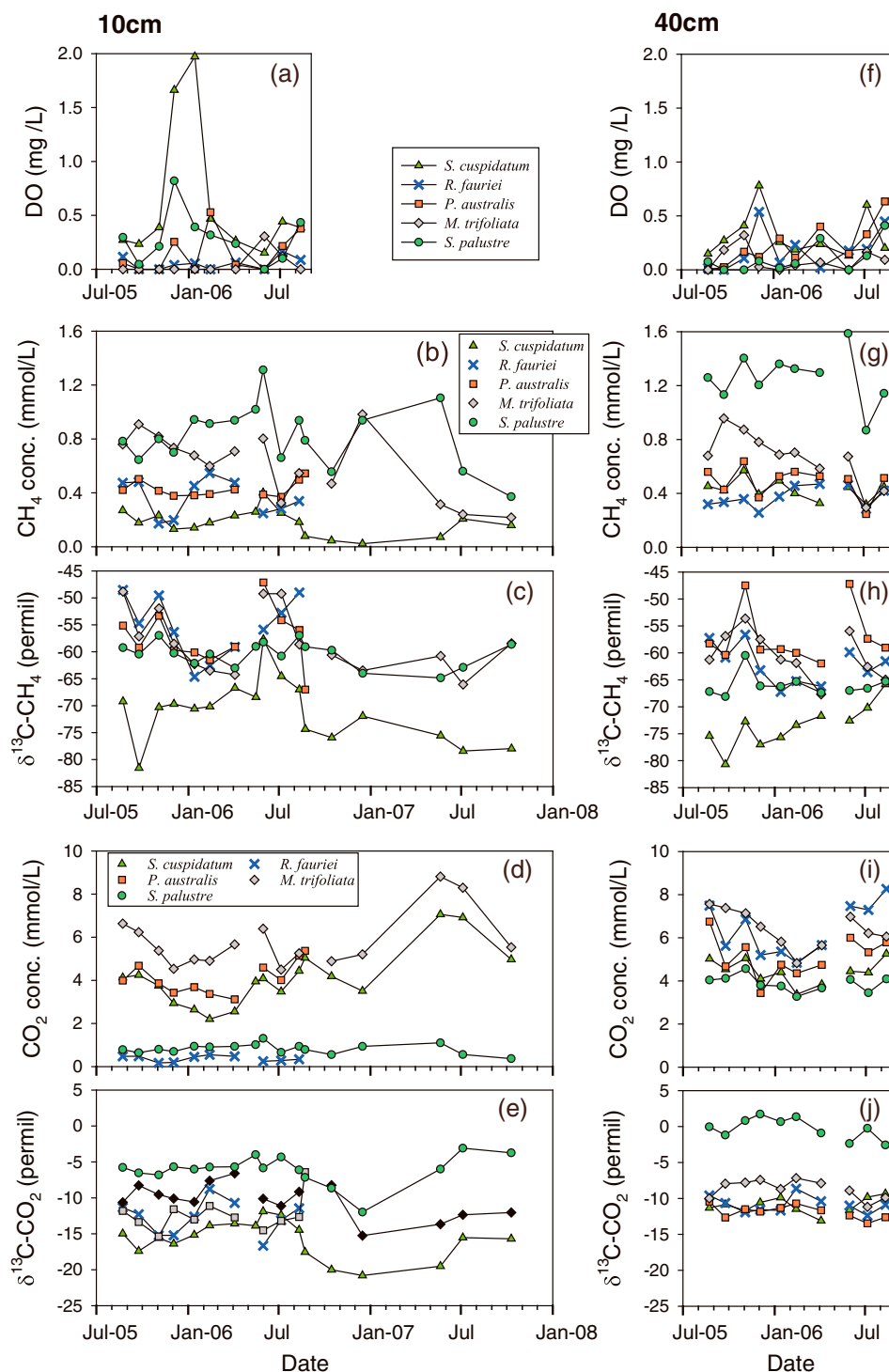
$-24.3 \pm 0.2\text{‰}$  (standard error (SE)),  $-28.5\text{‰}$ ,  $-25.1\text{‰}$ ,  $-27.4 \pm 0.2\text{‰}$  (standard error (SE)), and  $-27.3 \pm 0.5\text{‰}$  (SE) for *S. cuspidatum*, *R. fauriei*, *P. australis*, *M. trifoliata*, and *S. palustre*, respectively.

DO was lower during higher-temperature periods at the *Sphagnum* sites. Figure 5 shows the differences in mean ( $\pm$  SE) values of DO (Figures 4a and 4f). DO values at the *S. cuspidatum* sites were significantly higher than those at the *R. fauriei* ( $t > 3.9$ ,  $p < 0.002$ ), *P. australis* ( $t > 3.3$ ,  $p < 0.014$ ), and *M. trifoliata* ( $t > 4.0$ ,  $p < 0.002$ ) sites at the 10 cm depth (Figure 5a). A significant difference was observed at 40 cm between the *S. cuspidatum* site and the *M. trifoliata* site ( $t > 3.0$ ,  $p < 0.03$ , Figure 5a). At the *R. fauriei* and *M. trifoliata* sites, which were situated at the center of the floating mat (hollow), DO at the 10 cm depth was lower than that in other parts of the mat (Figure 5a).

### 3.2. Dissolved $\text{CH}_4$ and $\text{CO}_2$ Concentrations and $\delta^{13}\text{C}$ Values in Pore Water

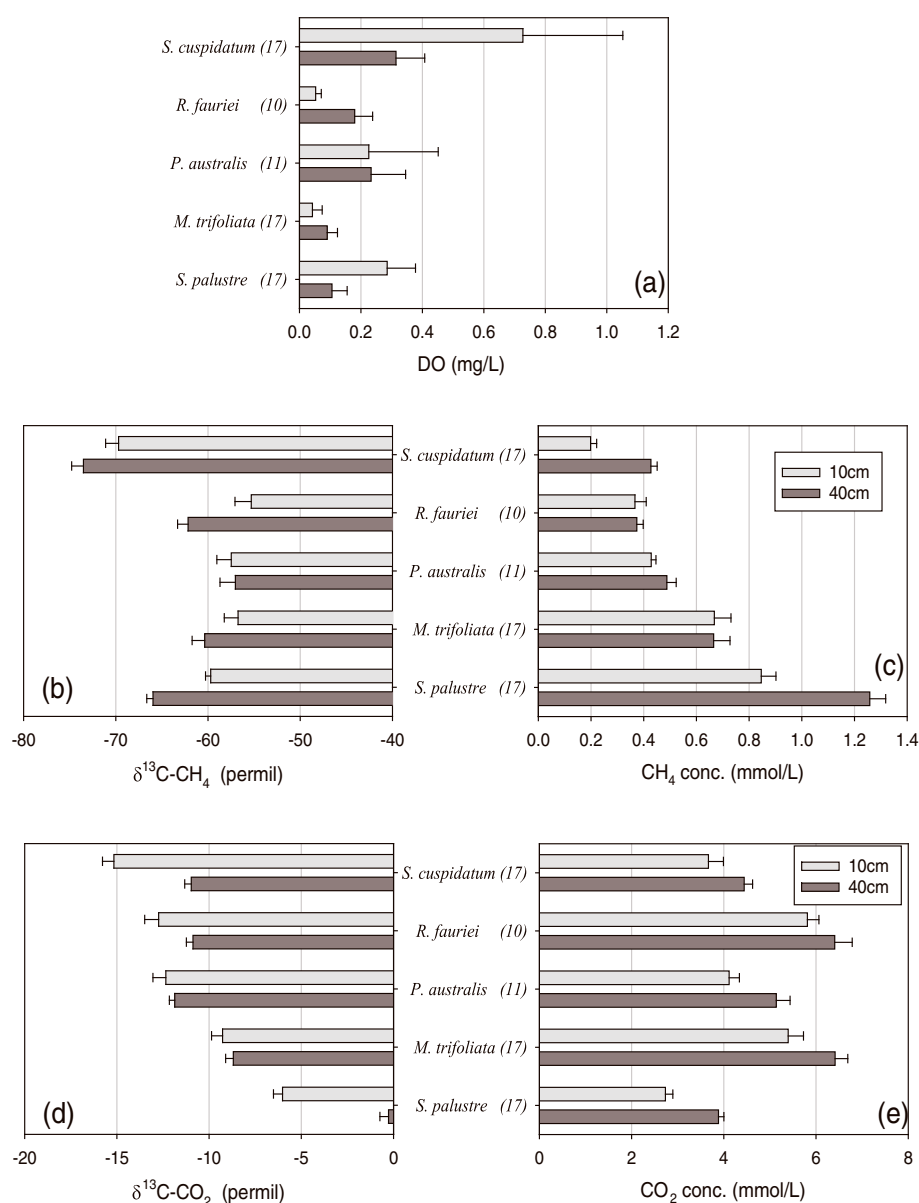
Generally, dissolved  $\text{CH}_4$  and  $\text{CO}_2$  concentrations increased in the summer for most of the sites (Figures 4b, 4d, 4g, and 4i). The  $\text{CH}_4$  concentration for the *P. australis* (reed) sites (10 cm, range 0.37–0.54 mmol/L, mean 0.38 mmol/L; 40 cm, range 0.25–0.64 mmol/L, mean 0.49 mmol/L) was generally stable throughout the sampling period (Figure 4b). Among the sites, the  $\text{CH}_4$  concentration from the *S. cuspidatum* sites at the 10 cm depth (range 0.02–0.40 mmol/L, mean 0.18 mmol/L) was significantly lower than that from the *P. australis* ( $t = 3.6$ ,  $p < 0.001$ ), *M. trifoliata* (range 0.22–0.98 mmol/L, mean 0.67 mmol/L;  $t = 6.8$ ,  $p < 0.001$ ), and *S. palustre* sites (range 0.37–1.31 mmol/L, mean 0.82 mmol/L;  $t = 10.6$ ,  $p < 0.001$ ) (Figure 5c and Table 2). In contrast, the  $\text{CH}_4$  concentration from the *S. palustre* habitats was significantly higher than that from any other habitat type at both depths ( $t > 3.4$ ,  $p < 0.009$  for 10 cm depth;  $t > 9.4$ ,  $p < 0.0001$  for 40 cm depth) (Figure 5c and Table 2).  $\text{CO}_2$  concentration was significantly different at each site compared with other sites at the same sampling depths (Figure 5e and Table 2). However, no significant differences were found ( $p > 0.05$ ) between the *S. cuspidatum* and *P. australis* sites at both depths, between *M. trifoliata* and *S. palustre* at both depths, and between *S. cuspidatum* and *S. palustre* at the 40 cm depth (Figure 5e and Table 2). The concentrations of both gases were higher at 40 cm than at 10 cm, and the variations were smaller at 40 cm (Figures 5c and 5e and Table 2).

Variations in  $\delta^{13}\text{C}\text{-CH}_4$  were species specific.  $\delta^{13}\text{C}\text{-CH}_4$  for the *S. cuspidatum* sites varied widely and was significantly more negative (10 cm, range  $-81.6$  to  $-57.7\text{‰}$ , mean  $-71.2\text{‰}$ ; 40 cm, range  $-80.7$  to  $-65.8\text{‰}$ , mean  $-73.5\text{‰}$ ,  $t > 6.2$ ,  $p < 0.0001$ ; Figure 4c, 4h, and 5b and Table 2) than for any other habitat type at both sampling depths (*R. fauriei*: 10 cm, range  $-64.6$  to  $-48.5\text{‰}$ , mean  $-55.3\text{‰}$ ; 40 cm, range



**Figure 4.** Seasonal variations in (a and f) dissolved oxygen and (b and g) dissolved CH<sub>4</sub> concentrations, (c and h)  $\delta^{13}\text{C-CH}_4$  values, (d and i) dissolved CO<sub>2</sub> concentrations, and (e and j)  $\delta^{13}\text{C-CO}_2$  values at depth of 10 and 40 cm, respectively.

–67.2 to –56.6‰, mean –62.2‰; *P. australis*: 10 cm, range –67.0 to –47.2‰, mean –55.3‰; 40 cm, range –62.0 to –47.2‰, mean –57.0‰; *M. trifoliata*: 10 cm, range –66.1 to –48.8‰, mean –56.7‰; 40 cm, range –67.7 to –53.6‰, mean –60.4‰; and *S. palustre*: 10 cm, range –64.8 to –56.9‰, mean –60.4‰, 40 cm; range –68.1 to –60.5‰, mean –66.0‰, respectively).  $\delta^{13}\text{C-CO}_2$  also increased in summer at most sites at 10 cm, and variations were small at 40 cm (Figures 4e, 4j, and 5d).  $\delta^{13}\text{C-CO}_2$  values at the *S. cuspidatum*



**Figure 5.** Vertical distributions of averaged ( $\pm$ SE) (a) dissolved oxygen, (b)  $\delta^{13}\text{C-CH}_4$ , (c)  $\text{CH}_4$  concentration, (d)  $\delta^{13}\text{C-CO}_2$  values, and (e) dissolved  $\text{CO}_2$  concentration for each hydrophyte type.

(10 cm: range  $-20.8$  to  $-11.9\text{‰}$ , mean  $-15.8\text{‰}$ ; 40 cm: range  $-13.1$  to  $-9.3\text{‰}$ , mean  $-11.0\text{‰}$ ) and *S. palustre* (10 cm: range  $-12.0$  to  $-3.1\text{‰}$ , mean  $-6.1\text{‰}$ ; 40 cm: range  $-2.6$  to  $1.7\text{‰}$ , mean  $-0.3\text{‰}$ ) sites were significantly more negative ( $t > 3.1$ ,  $p < 0.03$ ) and more positive ( $t > 5.2$ ,  $p < 0.0001$ ), respectively, than those at any other habitat type at 10 cm (Figure 5d and Table 2).  $\delta^{13}\text{C-CO}_2$  values in the *S. palustre* habitats at 40 cm were significantly more positive than those in any other habitat type ( $t > 15.6$ ,  $p < 0.0001$ , Figure 5d).

### 3.3. $\text{CH}_4$ Flux

$\text{CH}_4$  emissions at the *S. cuspidatum* sites were consistently low throughout the year (Figure 6). In contrast,  $\text{CH}_4$  emissions at the *M. trifoliata* site were stable at high levels throughout the year. Although we had only five flux sampling occasions, our results show that  $\text{CH}_4$  flux at the *M. trifoliata* site was significantly larger than that at the *Sphagnum* sites (*S. cuspidatum*,  $p < 0.001$  and *S. palustre*,  $p < 0.005$ ), consistent with the flux data of Sugimoto and Fujita [1997]. The amount of  $\text{CH}_4$  emissions we observed at the *M. trifoliata* site

**Table 2.** Mean CH<sub>4</sub> and CO<sub>2</sub> Concentrations and Mean, Minimum, and Maximum Values of  $\delta^{13}\text{C-CH}_4$  and  $\delta^{13}\text{C-CO}_2$  at Each Sampling Site and Depth

Plot	Depth (cm)	Mean ( $\pm$ SE) CH <sub>4</sub> Concentration (mmol/L)	$\delta^{13}\text{C-CH}_4$ (‰)			Mean ( $\pm$ SE) CO <sub>2</sub> Concentration (mmol/L)	$\delta^{13}\text{C-CO}_2$ (‰)			n
			mean ( $\pm$ SE)	min	max		mean ( $\pm$ SE)	min	max	
<i>S. cuspidatum</i>	10	0.18 $\pm$ 0.0	-71.2 $\pm$ 1.4	-81.6	-57.7	3.67 $\pm$ 0.3	-15.2 $\pm$ 0.6	-20.8	-11.9	17
	40	0.43 $\pm$ 0.0	-73.5 $\pm$ 1.3	-80.7	-65.8	4.44 $\pm$ 0.2	-11.0 $\pm$ 0.4	-13.1	-9.3	10
<i>R. fauriei</i>	10	0.37 $\pm$ 0.0	-55.3 $\pm$ 1.8	-64.6	-48.5	5.81 $\pm$ 0.3	-12.7 $\pm$ 0.8	-16.7	-8.9	10
	40	0.37 $\pm$ 0.0	-62.2 $\pm$ 1.1	-67.2	-56.6	6.41 $\pm$ 0.4	-10.9 $\pm$ 0.4	-12.4	-8.7	10
<i>P. australis</i>	10	0.43 $\pm$ 0.0	-57.5 $\pm$ 1.6	-67.0	-47.2	4.11 $\pm$ 0.2	-12.3 $\pm$ 0.7	-15.4	-6.4	11
	40	0.49 $\pm$ 0.0	-57.0 $\pm$ 1.7	-62.0	-47.2	5.14 $\pm$ 0.3	-11.9 $\pm$ 0.3	-13.5	-10.4	10
<i>M. trifoliata</i>	10	0.67 $\pm$ 0.1	-56.7 $\pm$ 1.5	-66.1	-48.8	5.40 $\pm$ 0.3	-9.3 $\pm$ 0.6	-15.3	-6.6	15
	40	0.67 $\pm$ 0.1	-60.4 $\pm$ 1.4	-67.7	-53.6	6.41 $\pm$ 0.3	-8.7 $\pm$ 0.4	-11.2	-7.2	10
<i>S. palustre</i>	10	0.82 $\pm$ 0.1	-60.4 $\pm$ 0.6	-64.8	-56.9	2.73 $\pm$ 0.2	-6.1 $\pm$ 0.5	-12.0	-3.1	17
	40	1.26 $\pm$ 0.1	-66.0 $\pm$ 0.7	-68.1	-60.5	3.88 $\pm$ 0.1	-0.3 $\pm$ 0.5	-2.6	1.7	10

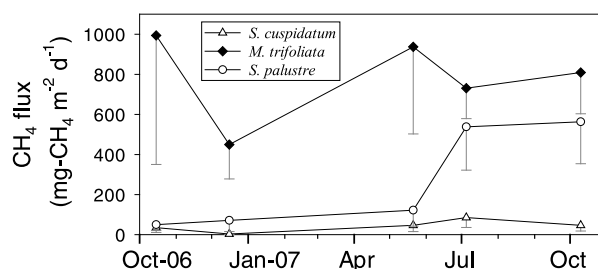
(ranging from 449.8 to 994.2 mg CH<sub>4</sub> m<sup>-2</sup> d<sup>-1</sup>) was similar to that observed at the *M. trifoliata* site in their report. CH<sub>4</sub> emissions at the *S. palustre* sites increased in the summer 2007 (Figure 6). CH<sub>4</sub> emissions at the *S. palustre* sites were one order of magnitude higher than that at *S. cuspidatum* sites on average (Figure 6).

## 4. Discussion

### 4.1. Differences in Redox Conditions and Their Effects on CH<sub>4</sub> and CO<sub>2</sub> Production

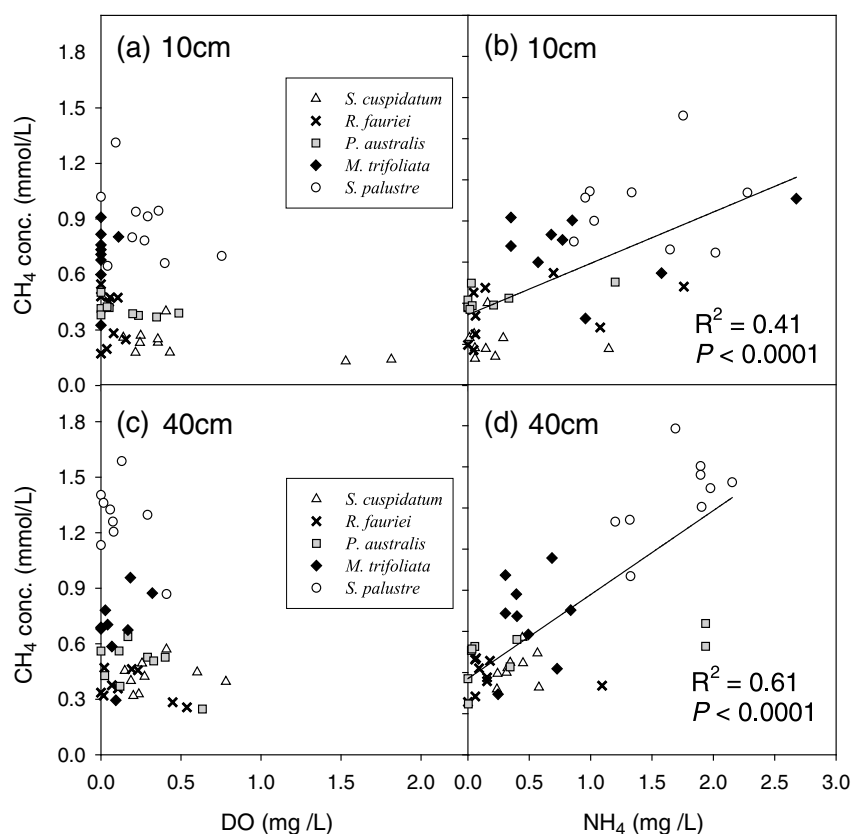
Low NO<sub>3</sub><sup>-</sup> and SO<sub>4</sub><sup>2-</sup> concentrations at all sampling sites indicate the existence of highly reduced conditions, in which existing NO<sub>3</sub><sup>-</sup> and SO<sub>4</sub><sup>2-</sup> are consumed as substrates for denitrification and sulfate reduction (Table 1). Figure 7 shows the relationships between DO and NH<sub>4</sub><sup>+</sup> concentrations versus CH<sub>4</sub> concentrations. Low CH<sub>4</sub> concentrations were observed only when DO was high (Figures 7a and 7c). In addition, significant positive relationships between NH<sub>4</sub><sup>+</sup> and CH<sub>4</sub> concentrations indicate that CH<sub>4</sub> production was higher under highly reduced conditions in which NH<sub>4</sub><sup>+</sup> consumption by nitrification was not prevalent (Figures 7b and 7d).

The *S. cuspidatum* habitats maintained higher DO concentrations than any other habitat type (Figures 4a and 4f). The characteristic location of *S. cuspidatum* habitats at the boundary of the water surface (Figure 3) must contribute to the high DO content, as oxygen is created by photosynthesis in the surface water. This oxic condition in *S. cuspidatum* habitats should suppress CH<sub>4</sub> production in shallow peat; therefore, CH<sub>4</sub> concentrations beneath *S. cuspidatum* habitats were maintained at lower levels than in other habitats. *S. palustre* sites on hummocks exhibited different results compared to *S. cuspidatum* sites, with significantly lower DO concentrations and higher CH<sub>4</sub> concentrations at both sampling depths (Figures 5a and 5c and Table 2). Accumulated *Sphagnum* tissues form dark conditions below the hummock surface. This contributed to prevent photosynthesis below the *Sphagnum* surface. Therefore, a highly reduced condition was maintained at the water surface without a supply of oxygen, and much CH<sub>4</sub> was produced. The CO<sub>2</sub> concentration at 10 cm for *S. palustre* habitats was significantly lower than for the other habitat types ( $p < 0.05$  for the *P. australis* sites and  $p < 0.001$  for the other habitats, Figure 5e and Table 2). It is generally known that *Sphagnum* tissue decomposes very slowly (reviewed by Van Breemen [1995]) because it produces humic acid [Karunen and Ekman, 1982; Rudolph and Samland, 1985] and polysaccharides [Clymo, 1963].



**Figure 6.** Seasonal variations in CH<sub>4</sub> flux from peat surfaces (with standard error).

Therefore, production of CH<sub>4</sub> and CO<sub>2</sub> was thought to be low. Among the *Sphagnum* species, Johnson and Damman [1991] reported that *S. cuspidatum* in hollows decayed 1.5 times faster than *S. fuscum*, which occupied hummocks. In addition, Hogg [1993] compared decay potential (CO<sub>2</sub> emission from bog organic matter from *Sphagnum*) in hollow and hummock species and reported that CO<sub>2</sub> emission rates were significantly higher in



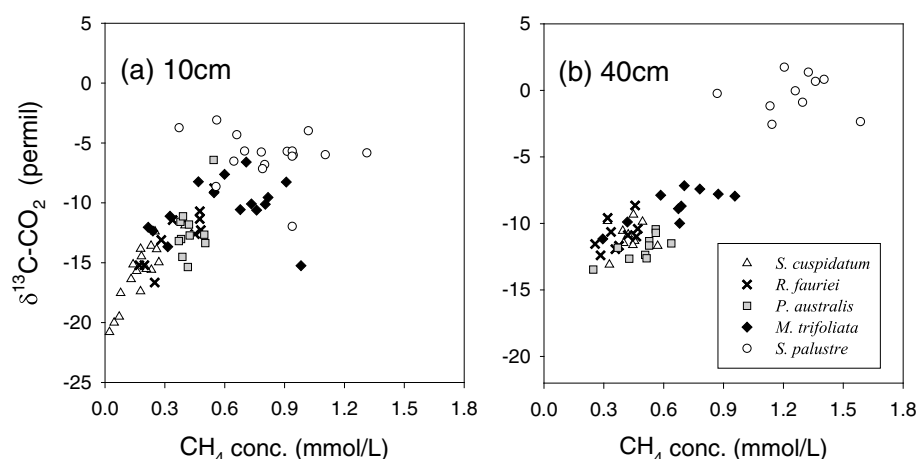
**Figure 7.** (a and c) Relationships between dissolved oxygen and (b and d) ammonium concentration, and CH<sub>4</sub> concentration.

hollows in deeper (50–100 cm) layers than those on hummocks. Our results showed results similar to their reports, that is, higher CO<sub>2</sub> concentration in pore water from *S. cuspidatum* habitats (hollows) than in that from *S. palustre* habitats (hummocks). This was supported by significantly higher DOC/DON ratio in *S. palustre* habitats than in *S. cuspidatum* habitats, which suggests DOC in *S. palustre* habitats was less labile (Table 1). In addition to these data on CO<sub>2</sub> conditions in both *Sphagnum* habitats, our results add information on CH<sub>4</sub> dynamics in *Sphagnum* species both in hollows and on hummocks. We showed that even though both *S. cuspidatum* and *S. palustre* belong to the same genus, different redox conditions are formed, and CH<sub>4</sub> concentrations in their habitats are also different. This also possibly reflects the contribution of CH<sub>4</sub> production from H<sub>2</sub>/CO<sub>2</sub> to the lower CO<sub>2</sub> concentrations in *S. palustre* sites.

Recently, CH<sub>4</sub> oxidation in *Sphagnum* mosses was examined, and Raghoebarsing *et al.* [2005] and Kip *et al.* [2010] found that CH<sub>4</sub> oxidation was most pronounced in submerged mosses. <sup>13</sup>C labeling revealed that CH<sub>4</sub>-derived carbon was incorporated into plant lipids when moss was submerged, indicating a mutually beneficial symbiosis between *Sphagnum* mosses and methanotrophs [Kip *et al.*, 2010]. In addition, Larmola *et al.* [2010] observed 41 *Sphagnum* species and found that all 41 species exhibited methanotrophic activity, and potential CH<sub>4</sub> oxidation was high when the water table level was near the surface. We observed lower CH<sub>4</sub> concentrations in the *S. cuspidatum* habitats (hollows) than in the *S. palustre* habitats (hummocks), which may be attributable to this methanotrophic activity under submerged conditions. More positive  $\delta^{13}\text{C-CH}_4$  values at shallower depths in both *Sphagnum* habitats also support the CH<sub>4</sub> oxidation and its preferential use of <sup>12</sup>CH<sub>4</sub>.

At the center of the floating mat, where the *R. fauriei* and *M. trifoliata* habitats were located (Figure 3), DO was maintained at low levels at 10 cm (Figure 4a). At these sites, CO<sub>2</sub> concentrations were significantly higher than at the other sites ( $p < 0.001$ ) and were similar in these two habitats (Figure 5e). Sugimoto and Fujita [1997] conducted an incubation experiment (measurement of gas production) to discriminate the decomposition





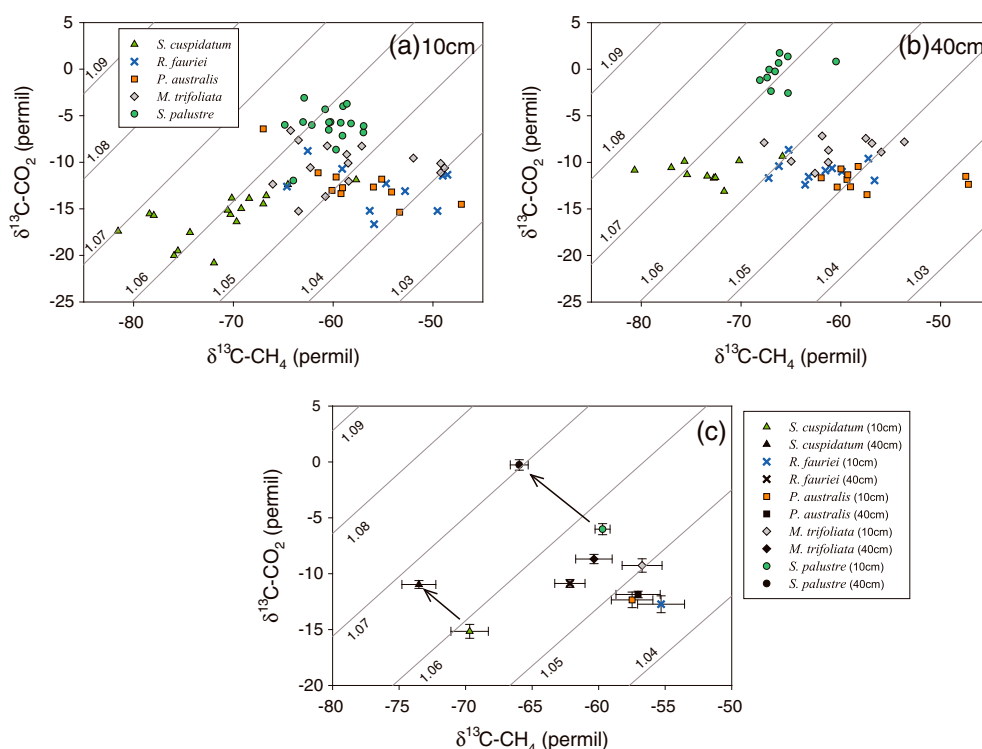
**Figure 8.** Relationships between  $\text{CH}_4$  concentration and  $\delta^{13}\text{C-CO}_2$  values at (a) 10 cm and (b) 40 cm.

rates of peat at *M. trifoliata*, *P. australis*, and *Sphagnum* habitats and found that the decomposition rate was in the order of *M. trifoliata* > *P. australis* > *Sphagnum*. In their experiments, the gas production rate of the surface peat at *M. trifoliata* site was almost 4 times as great as that at 40 cm depth. We consider that high  $\text{CO}_2$  concentrations at *M. trifoliata* sites in our observations can be explained by this. Lower DOC/DON ratio at the *M. trifoliata* site at 10 cm depth compared with the other sites suggests the existence of more labile carbon in shallow part of these sites (Table 1). These must contribute higher  $\text{CH}_4$  production, which explains the fact that the  $\text{CH}_4$  concentration at 10 cm was significantly higher at the *M. trifoliata* site than at the *R. fauriei* site ( $p < 0.05$ ), although these habitats were situated adjacently in the center of the mat (Figure 5c). The presence of the lowest DO and the highest  $\text{CO}_2$  concentration at 10 cm in these habitats indicates that anoxic respiration producing  $\text{CO}_2$  was dominant in this area. The DO at 40 cm was moderately and greatly higher than that at 10 cm in *M. trifoliata* and *R. fauriei* habitats, respectively (Figure 5a). Considering that the root depth of *M. trifoliata* is not deeper than 10 cm, more decomposition of labile organic matter at 10 cm than at 40 cm in the *M. trifoliata* habitat [Sugimoto and Fujita 1997] can contribute to the formation of such conditions. The larger root depth of *R. fauriei* also could transport oxygen to the deeper layer.

At the edges of the mat, where *P. australis* (reeds) grew, DO,  $\text{CH}_4$ , and  $\text{CO}_2$  concentrations were at intermediate levels compared to most of the sampling sites (Figure 5). This agreed with the report of Sugimoto and Fujita [1997] that decomposition rates of reeds were intermediate between *M. trifoliata* and *Sphagnum*. They also reported that considerably higher decomposition rates were detected at 10 cm than at 40 cm at the reed site. However, our results showed that the  $\text{CH}_4$  and  $\text{CO}_2$  concentrations were higher at 40 cm depth than at 10 cm depth. This can probably be attributed to gas exchange between pore water and the air at shallower zones and/or homogenization of dissolved gas by exchanging pore water and pond water because this habitat is situated at the edges of the floating mat (Figure 3).

#### 4.2. Implications From the $\delta^{13}\text{C}$ Ratios of $\text{CH}_4$ and $\text{CO}_2$

Figure 8 shows the clear relationship between  $\text{CH}_4$  concentration and  $\delta^{13}\text{C-CO}_2$ . The distributions of the plots of each species were highly specific; that is, the lowest  $\text{CH}_4$  concentrations and the most negative  $\delta^{13}\text{C-CO}_2$  were observed at *S. cuspidatum* habitats, and highest  $\text{CH}_4$  concentrations and the most positive  $\delta^{13}\text{C-CO}_2$  were observed at *S. palustre* habitats. The other species were situated between the two *Sphagnum* species. Such a relationship between  $\text{CH}_4$  concentration and  $\delta^{13}\text{C-CO}_2$  was reported for seasonal variations of the pore water samples obtained from a Japanese forested wetland under the same climatic conditions [Itoh et al., 2008]. The authors suggested that the  $\delta^{13}\text{C-CO}_2$  increase in correlation with increasing  $\text{CH}_4$  concentrations in high-temperature periods was due to the process of the preferential use of  $^{12}\text{C}$  as a substrate for  $\text{CH}_4$  by  $\text{H}_2/\text{CO}_2$  reduction [Sugimoto and Wada, 1993]. In our floating bog site, the significant positive regression of  $\text{CH}_4$  concentration on  $\delta^{13}\text{C-CO}_2$  (Figure 8) was not similar to the results of Itoh et al. [2008] in which the significant positive regression was shown as a seasonal variation in one plot. At our site, a significant positive regression of  $\text{CH}_4$  concentration on  $\delta^{13}\text{C-CO}_2$



**Figure 9.** Crossplots of  $\delta^{13}\text{C}$  data from  $\text{CH}_4$  and  $\text{CO}_2$  for each habitat type at (a) 10 cm (a), (b) 40 cm, and (c) averaged ( $C_i \pm \text{SE}$ ). The dashed lines indicate the apparent fractionation between  $\text{CO}_2$  and  $\text{CH}_4$  ( $\alpha$ ) calculated by the ratio of  $(\delta^{13}\text{C}\text{-CO}_2 + 10^3)/(\delta^{13}\text{C}\text{-CH}_4 + 10^3)$ .

was observed but as a hydrophyte species-specific distribution in the  $\text{CH}_4$  concentration— $\delta^{13}\text{C}\text{-CO}_2$  diagram. The measured plant tissue  $\delta^{13}\text{C}$  values in our site ranged from  $-24.3$  to  $-28.5\text{‰}$ . Considering that  $\text{CO}_2$  is produced as a byproduct of decomposition of these plant tissues or peats under both oxic and anoxic conditions,  $\text{CH}_4$  production must contribute high values of  $\delta^{13}\text{C}\text{-CO}_2$ . These results show that preferential reduction of  $^{12}\text{CO}_2$  for methanogenesis can drastically change  $\delta^{13}\text{C}\text{-CO}_2$ . Under the condition that hydrogen concentration increases in the summer in this floating mat [Sugimoto and Fujita, 2006], it has been suggested that  $\text{H}_2/\text{CO}_2$  reduction makes a considerable contribution to  $\text{CH}_4$  production, especially in the summer.

The  $\text{CH}_4$  concentration was lower and  $\delta^{13}\text{C}\text{-CO}_2$  was more negative at 10 cm depth than at 40 cm in all habitats ( $\text{CH}_4$ ,  $p < 0.001$  for both *Sphagnum* habitats;  $\delta^{13}\text{C}\text{-CO}_2$ ,  $p < 0.001$  for both *Sphagnum* habitats; and  $p < 0.05$  for the other three habitats). This indicates the possibility of preferential use of  $^{12}\text{CH}_4$  by methanotrophs in shallower layer, which produce more negative  $\text{CO}_2$ . This is also supported by  $\delta^{13}\text{C}\text{-CH}_4$  values, as discussed in the following.

The crossplots of  $\delta^{13}\text{C}\text{-CH}_4$  and  $\delta^{13}\text{C}\text{-CO}_2$  are shown in Figure 9, with the apparent fractionation between  $\text{CO}_2$  and  $\text{CH}_4$  ( $\alpha$ ) calculated based on the ratio  $(\delta^{13}\text{C}\text{-CO}_2 + 1000)/(\delta^{13}\text{C}\text{-CH}_4 + 1000)$ . The values of  $\alpha$  indicate the magnitude of isotopic separation between coexisting species. The apparent  $\alpha$  distribution ranged from 1.034 to 1.073 at our site (Table 3). Whiticar et al. [1986] suggest that  $\text{CH}_4$  produced primarily by the acetate fermentation pathway in freshwater environments has  $\delta^{13}\text{C}$  values ranging from  $\sim -65$  to  $-50\text{‰}$ , whereas  $\text{CH}_4$  produced primarily by the  $\text{CO}_2$  reduction pathway in marine systems has  $\delta^{13}\text{C}$  values ranging from  $\sim -110$  to  $-60\text{‰}$ . Also, from incubation studies,  $^{13}\text{C}$  fractionation during  $\text{CO}_2$  reduction to  $\text{CH}_4$  ( $1.025 \leq \alpha_{\text{CO}_2\text{-CH}_4} \leq 1.079$ ) is larger than that associated with acetate dissimilation ( $\alpha_{\text{acetate-CH}_4} \leq 1.021$ ) [Games et al., 1978; Gelwicks et al., 1994; Botz et al., 1996]. The values of  $\alpha$  at our sites varied from species to species; that is, wide ranges of values were observed at vascular plant sites and narrow ranges of  $\alpha$  values in high- $\alpha$  zone at *S. cuspidatum* and *S. palustre* sites (Figure 9c and Table 3). High  $\delta^{13}\text{C}\text{-CO}_2$  values were observed at highly reduced

**Table 3.** Mean, Minimum, and Maximum Values of  $\alpha$  at Each Sampling Site and Depth

Plot	Depth (cm)	$\alpha$		
		Mean	Min	Max
<i>S. cuspidatum</i>	10	1.059	1.049	1.070
	40	1.068	1.060	1.076
<i>R. fauriei</i>	10	1.045	1.036	1.057
	40	1.055	1.048	1.064
<i>P. australis</i>	10	1.047	1.034	1.065
	40	1.048	1.037	1.054
<i>M. trifoliata</i>	10	1.050	1.040	1.062
	40	1.055	1.047	1.061
<i>S. palustre</i>	10	1.057	1.053	1.064
	40	1.070	1.065	1.073

sites where  $\text{CH}_4$  was produced actively (*S. palustre* and *M. trifoliata* sites) and vice versa at oxic sites (*S. cuspidatum* sites). From this, we can assume that the range of  $\delta^{13}\text{C}-\text{CO}_2$  for each species in the  $\delta^{13}\text{C}-\text{CH}_4-\delta^{13}\text{C}-\text{CO}_2$  diagram was determined by the redox condition of the habitat.

Higher  $\alpha$  at *S. cuspidatum* and *S. palustre* sites (Figure 9c) indicates that a larger portion of  $\text{CH}_4$  originated from  $\text{H}_2/\text{CO}_2$  reduction in the two *Sphagnum* habitat types, although the degree of methanogenic activity differed. These results agree with

the reports of Hornibrook and Bowes [2007] and Hornibrook [2009], in which  $\delta^{13}\text{C}-\text{CH}_4$  values in pore water were more positive in minerotrophic peatland than in ombrogenous peatland, and of Galand et al. [2005], in which a larger portion of  $\text{CH}_4$  from  $\text{H}_2/\text{CO}_2$  was observed from ombrotrophic bog than from the mesotrophic fen in community studies of methanogen. These suggest that  $\delta^{13}\text{C}-\text{CH}_4$  values in a peatland can be shifted with trophic status.

When considering the change in  $\alpha$  with depth, increasing  $\alpha$  values with depth as a result of decreasing  $\delta^{13}\text{C}-\text{CH}_4$  and increasing  $\delta^{13}\text{C}-\text{CO}_2$  were observed at most sites, with the exception of the *P. australis* sites (in the same manner as indicated by the arrows in Figure 9c at the *Sphagnum* sites) (Table 3). Such shifts in  $\alpha$  values with increasing depth were reported as the effect of increasing  $\text{CH}_4$  from  $\text{H}_2/\text{CO}_2$  reduction pathway relative to acetate fermentation [Hornibrook et al., 1997, 2000]. They suggested that, in shallow soils containing an abundance of labile organic carbon, the contribution of the acetate fermentation pathway to  $\text{CH}_4$  production was relatively high and that a shift occurred toward carbonate-utilizing methanogenesis with increasing depth as organic matter became increasingly recalcitrant. Such observations that acetoclastic methanogenesis occurs preferably in the upper peat layer and that  $\text{CH}_4$  production from  $\text{H}_2/\text{CO}_2$  dominates in the deep layers have been previously reported [Popp et al., 1999; Chasar et al., 2000; Kotsyurbenko et al., 2004]. Miyajima et al. [1997] also demonstrated that a decrease in the degradability of organic matter resulted in an enhanced contribution of  $\text{CH}_4$  from the  $\text{H}_2/\text{CO}_2$  reduction pathway. Our results suggest that the same direction of shift in methanogenic pathway with depth can be found within each hydrophyte habitat, especially in *Sphagnum* habitats.

In addition,  $\delta^{13}\text{C}-\text{CH}_4$  was more positive at shallower (10 cm) than at greater (40 cm) depth, whereas  $\delta^{13}\text{C}-\text{CO}_2$  was more negative at the shallower depth at both *Sphagnum* sites. This may be a result of  $\text{CH}_4$  oxidation in *Sphagnum* habitats (as discussed above). Coleman et al. [1981] reported that the carbon isotope effect of  $\text{CH}_4$ -oxidizing bacteria ranged from 1.013 to 1.025 in culture experiments, and Tyler et al. [1994] reported a similar value of  $1.022 \pm 0.004$  from temperate forest soil. Therefore,  $\delta^{13}\text{C}$  values of  $\text{CO}_2$  produced by  $\text{CH}_4$  oxidation will have more negative values compared with those of respired  $\text{CO}_2$  from other sources. Considering this, more positive  $\delta^{13}\text{C}-\text{CH}_4$  values and more negative  $\delta^{13}\text{C}-\text{CO}_2$  values at shallower depths in *Sphagnum* habitats can be partly explained by  $\text{CH}_4$  oxidation by methanotrophs (Table 4).

As for habitats other than the *Sphagnum* sites,  $\delta^{13}\text{C}-\text{CH}_4$  values were significantly more negative in the deeper zone (40 cm) than in the shallower zone (10 cm) in *R. fauriei* habitat ( $t > 2.2$ ,  $p < 0.05$ ). Chanton [2005], summarizing the  $\delta^{13}\text{C}-\text{CH}_4$  values of diffusive plants (e.g., *Peltandra* and *Oryza*), reported that more negative  $\text{CH}_4$  is transported and emitted to the atmosphere via plants, and more positive  $\text{CH}_4$  is retained in the sediment. Considering that the roots of *R. fauriei* do not reach as deep as 40 cm, more positive  $\delta^{13}\text{C}-\text{CH}_4$  values at 10 cm depth were thought to be the result of  $\text{CH}_4$  diffusion via plants and/or  $\text{CH}_4$  oxidation in the rhizosphere. This agrees with the report by Popp et al. [1999], who measured the  $\delta^{13}\text{C}-\text{CH}_4$  in a *Carex* fen and characterized emitted  $\text{CH}_4$ , rhizospheric  $\text{CH}_4$ , and  $\text{CH}_4$  below the rhizosphere. They reported that emitted  $\text{CH}_4$  was isotopically similar to  $\text{CH}_4$  below the rhizosphere (at 50 cm depth), whereas  $\text{CH}_4$  within the

**Table 4.** The Slope at Each Sampling Site for the Crossplots of  $\delta^{13}\text{C}\text{-CH}_4$  Versus  $\delta^{13}\text{C}\text{-CO}_2$  Between 10 and 40 cm Depths (Figure 9c)

Site	Slope of $\delta^{13}\text{C}\text{-CH}_4$ Versus $\delta^{13}\text{C}\text{-CO}_2$
<i>S. cuspidatum</i>	−1.10
<i>R. fauriei</i>	−0.27
<i>P. australis</i>	1.08
<i>M. trifoliata</i>	−0.16
<i>S. palustre</i>	−0.92

rhizosphere (5–20 cm) was relatively  $^{13}\text{C}$  enriched. This suggests that more enriched  $\text{CH}_4$  in the rhizosphere is due to oxidation and transport via plants and that  $\text{CH}_4$  at 50 cm is below the bulk of the root zone and free from these effects. At the *M. trifoliata* site,  $\text{CH}_4$  concentration was almost similar at the 10 and 40 cm depths,

and  $\delta^{13}\text{C}\text{-CH}_4$  was slightly more positive at the 10 than at the 40 cm depth. This suggests that the effect of  $\text{CH}_4$  transport and  $\text{CH}_4$  oxidation in the rhizosphere could not have been detected from the pore water at 10 cm depth in the *M. trifoliata* site, probably because of much shallower root depth (approximately 5 cm) compared with *R. fauriei*. Both  $\text{CH}_4$  concentration and  $\delta^{13}\text{C}\text{-CH}_4$  values were similar at both the 10 and 40 cm depths at the *P. australis* (reed) sites. This is also in agreement with Chanton [2005], who showed little difference in  $\delta^{13}\text{C}\text{-CH}_4$  in sediment  $\text{CH}_4$  and  $\text{CH}_4$  emitted during the daytime for convective plants (e.g., *Typha* and *Phragmites*). They suggested that the convective through-flow system under daylight does not result in molecular-weight-dependent fractionation of  $\text{CH}_4$ . The fact that rhizomes of reeds can grow below 40 cm in depth also contributes to the homogeneous distribution of  $\text{CH}_4$  and  $\delta^{13}\text{C}\text{-CH}_4$  down to the depth of 40 cm. From these results, our isotopic data also reflect the habitat-specific  $\text{CH}_4$  emission mechanisms at our site.

#### 4.3. Methane Production and Methane Flux

The fact that the *M. trifoliata* site had higher  $\text{CH}_4$  emissions than the *Sphagnum* sites confirms the previous results of Sugimoto and Fujita [1997]. This also agree with the results of Bowes and Hornibrook [2006], who reported that the  $\text{CH}_4$  flux was an order of magnitude less at a *Sphagnum*-rich site than at a vascular flora-rich site. The ability of vascular plants to enhance  $\text{CH}_4$  flux from wetland surfaces is well known [e.g., Thomas et al., 1996; Wassmann and Aulakh, 2000; Kutzbach et al., 2004].  $\text{CH}_4$  concentration at 10 cm depth was significantly higher at *S. palustre* sites than at *M. trifoliata* sites throughout the sampling period. Gas transport via *M. trifoliata* must contribute to larger  $\text{CH}_4$  emission in their habitat compared with the *S. palustre* habitat.  $\text{CH}_4$  oxidation within *Sphagnum* moss (as discussed above) can also contribute to this difference. In addition, lower dissolved  $\text{CH}_4$  concentrations (Figure 4b) and higher  $\text{CH}_4$  flux (Figure 6) were observed at the *M. trifoliata* site in the summer of 2007, when the mat was floating and the water table was below the soil surface, than in winter. This also supports the conclusion that the life form of *M. trifoliata* controls the seasonal pattern of  $\text{CH}_4$  flux.

Although the difference was not significant,  $\text{CH}_4$  emission remained higher at the *S. palustre* site than at the *S. cuspidatum* site, and much higher emissions were observed during summer. This must reflect the difference in dissolved  $\text{CH}_4$  beneath these habitats. At the *S. cuspidatum* site, a significant positive relationship was observed between dissolved  $\text{CH}_4$  concentrations in pore water at 10 cm and  $\text{CH}_4$  flux ( $n = 5$ ,  $F = 10.4$ ,  $p < 0.05$ ). In contrast, at the *S. palustre* site, no relationship was observed between dissolved  $\text{CH}_4$  at 10 cm and emitted  $\text{CH}_4$ , suggesting that accumulated peat can also affect the transport of  $\text{CH}_4$  produced beneath the *S. palustre* habitat. This also can induce isotopic fractionation in  $\delta^{13}\text{C}\text{-CH}_4$  values during  $\text{CH}_4$  transportation to the atmosphere. Hornibrook [2009] reported that  $\delta^{13}\text{C}$  values of emitted  $\text{CH}_4$  from ombrotrophic bogs were more negative than from fens reflecting the differences of  $\text{CH}_4$  production,  $\text{CH}_4$  oxidation, and transportation in relationship to trophic status including vegetation. Further study to understand the difference in the  $\delta^{13}\text{C}\text{-CH}_4$  values in emitted  $\text{CH}_4$  among *Sphagnum* habitats is also needed considering their life forms.

#### 5. Conclusions

Vegetation changes in peatlands will be accompanied by eutrophication and/or climate changes, such as increasing temperatures and higher frequencies of heavy precipitation in the future [e.g., Dai, 2013]. Our results from observations in a small floating bog peatland that has experienced eutrophication suggest that  $\text{CH}_4$  concentrations and  $\delta^{13}\text{C}\text{-CH}_4$  and  $\delta^{13}\text{C}\text{-CO}_2$  values in pore water varied greatly among the hydrophyte habitats. This suggests that different plant life forms can affect  $\text{CH}_4$  production by controlling redox conditions and determining  $\delta^{13}\text{C}\text{-CH}_4$  and  $\delta^{13}\text{C}\text{-CO}_2$  values, even within *Sphagnum* species. Differences in the amount of  $\text{CH}_4$  beneath hydrophyte habitats and their life forms can also affect the  $\text{CH}_4$

emission intensity. *Hornibrook and Bowes* [2007] and *Hornibrook* [2009] measured the  $\delta^{13}\text{C}\text{-CH}_4$  of both pore water and  $\text{CH}_4$  flux from two ombrogenous and two minerotrophic peatlands, focusing on the trophic status, which impacts  $\text{CH}_4$  production and emission via control of vegetation assemblages. They reported that the  $\delta^{13}\text{C}\text{-CH}_4$  of both pore water and  $\text{CH}_4$  flux was more negative in ombrogenous bogs than in minerotrophic peatlands. Taking into consideration the variety of hydrophytes growing in peatlands and their transitions can provide useful information for a better understanding of  $\text{CH}_4$  dynamics and its emission from peat bogs. Although we did not show the isotopic data for the  $\text{CH}_4$  flux in this paper, our results implied that considerable difference can be shown in  $\delta^{13}\text{C}\text{-CH}_4$  values in emitted  $\text{CH}_4$  from the different type of hydrophytes within a small floating peat bog.

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